Effects of RAS blockade on experimental chronic transplant failure

Smit-van Oosten, Annemieke

Summary

Chronic renal transplant failure (CRTF) is the predominant cause of late graft failure. One of the histological features in the transplanted kidney is intimal hyperplasia. In the present thesis, experiments are described studying vascular changes in CRTF. There still is no treatment to prevent CRTF. In other renal diseases, ACE inhibitors are used to prevent or slow down the progression of renal failure by normalizing proteinuria, reducing blood pressure and attenuating fibrosis. Therefore, in the second chapter, we studied the therapeutic effects of ACE inhibition on CRTF. We transplanted kidney grafts in the Fisher to Lewis allogeneic rat strain combination. These rats were subsequently treated with either an ACE inhibitor or an AT1 receptor blocker. Both treatments were shown to be beneficial with respect to proteinuria, hypercholesterolemia, and glomerulosclerosis. However, we found that long-term treatment of the allografted rats caused severe intimal hyperplasia in the transplanted organ. The cells present in the intima were of myofibroblastic origin. Other studies using ACE inhibition in experimental transplantation did not find this severe intimal hyperplasia or reported favourable effects of ACE inhibition on intimal thickness, but these studies lasted for a maximum of 20/24 weeks. Our data in rats in which treatment was started at a later time point suggested that intimal hyperplasia starts after 20 to 24 weeks of RAS blockade. Our study suggests that long-term absence of angiotensin II action results in renal vascular pathology. Other studies indeed show that growth factor production occurs not only at high levels of angiotensin II [1,2,3], but also when angiotensin II is absent [4].

In chapter 3 we tried to identify the mechanisms underlying the ACE inhibition induced intimal hyperplasia. First, to assess whether ACE inhibition induced vascular thickening is allospecific, we treated Fisher to Lewis allografts and syngrafts (Lewis to Lewis and Fisher to Fisher) with cyclosporin A and ACE inhibition. Second, to determine whether the effect is specific for the allocombination Fisher to Lewis, we now also used the Lewis to Fisher allograft combination, treated with cyclosporin A and ACE inhibition. Third, to determine if the combination cyclosporin A-ACE inhibition contributes to ACE inhibition induced intimal hyperplasia, we also included a group of allografted rats (Fisher to Lewis) which were not treated with cyclosporin A, but received ACE inhibition only. We found that chronic treatment with ACE inhibition in syngrafted or allografted rats with a Fisher kidney graft causes severe intimal hyperplasia, whereas the Lewis graft is protected in both combinations. Although ACE inhibition induced intimal hyperplasia depends on the donor kidney, it is significantly aggravated by allografting. The short course of cyclosporin A treatment did not contribute to ACE inhibition induced intimal hyperplasia. Studies in angiotensin knock out mice have revealed that these mice have vascular abnormalities and increased vascular expression of PDGF. In vitro studies have shown that smooth muscle cells of Fisher rats have an increased growth response to PDGF compared to Lewis rats [5]. This interstrain difference may also be involved in the development of intimal hyperplasia after long-term ACE inhibition in the Fisher kidney.

In chapter 4 we investigated strain-related differences in intrarenal ACE activity between Fisher and Lewis rats, since Fisher rats are more vulnerable to develop renal damage. In addition, we tested whether treatment with ACE inhibition in allografted rats results in reduction of intrarenal ACE. We found that Fisher rats have significantly higher ACE activity and ACE mRNA expression, in comparison with Lewis rats. In addition, using immunohistochemistry, we found that the Fisher rat has a strong tubular ACE expression, whereas the Lewis rat has no tubular ACE at all. This difference in ACE levels may have a genetic origin, similar to the ACE polymorphism in humans. The higher intrarenal ACE activity of the Fisher rat may explain the higher glomerular intracapillary pressures found in these rats, compared with Lewis rats [6]. In addition, these high ACE levels may render the Fisher rat more vulnerable to interstitial fibrosis and inflammation, since angiotensin II is a pro-inflammatory and fibrotic agent. Treatment of the allografted rats with ACE inhibition prevented proteinuria and glomerulosclerosis and reduced ACE activity with 58%. These findings suggest that a high intrarenal ACE status predisposes to transplantation related renal damage.

In chapter 5 we studied the presence of ischemic injury in the renal graft of allografted rats and the effect of ACE inhibition on ischemic injury. Down-regulation of the enzyme ecto-ATPase in combination with up-regulation of the enzyme ecto-5'-nucleotidase is a hallmark for ischemic damage. The untreated allografted rats revealed marked up-regulation of ecto-5'-nucleotidase in the renal vasculature and this was further enhanced by ACE inhibition. This staining pattern points to ischemic damage in the renal vasculature, the obliteration of renal arteries promotes an ischemic environment. Glomerular ecto-ATPase staining was reduced in untreated allografts, possibly due to ischemic damage, however, ACE inhibition prevented glomerulosclerosis and also prevented the reduction in ecto-ATPase staining. This renoprotective effect of ACE inhibition may depend upon the effect ACE inhibition has on renal interstitial oxygenation [7].

As cardiovascular disease is a major cause of death following renal transplantation, in chapter 6 the consequences of renal transplantation on the extrarenal vasculature of the recipient animal was studied in rat aortic rings. We found that NO pathways were not affected in syngrafted and allografted rats at 34 weeks after transplantation. However, the contribution of COX-derived prostaglandins in vascular function outside the transplanted organ is strongly diminished after allogeneic transplantation. RAS blockade fails to restore prostaglandin function, despite major improvements of the common cardiovascular risk factors in this model.

In conclusion, we found that ACE inhibition lowers blood pressure, reduces hypercholesterolemia and proteinuria and prevents glomerulosclerosis in the Fisher to Lewis allograft combination. However, long term treatment of the transplanted rats with ACE inhibition causes severe renal intimal hyperplasia in Fisher kidney grafts, which was aggravated by allografting. ACE inhibition prevents ischemic damage in the glomeruli, but worsens ischemic damage in the intrarenal vessels. The altered cardiovascular prostaglandin function outside the transplanted organ in the allografted rat could not be restored by ACE inhibition.